Sentinel Lymph Node Biopsy in Colon Cancer
A Prospective Multicenter Trial

Andreas E. Bembenek, MD, Robert Rosenberg, MD,† Elke Wagler, MD,‡ Stephan Gretschel, MD,* Andreas Sendler, MD,† Joerg-Ruediger Siewert, MD,† Jörg Nährig, MD,†† Helmut Witzigmann, MD,‡ Johann Hauss, MD,‡ Christian Knorr, MD,§ Arno Dimmler, MD,†† Jörn Gröne, MD,‖ Heinz-Johannes Buhr, MD,‖ Jörg Haier, MD,¶ Hermann Herbst, MD,§§§ Juergen Tepel, MD,# Bence Siphos, MD,‖‖‖ Axel Kleespies, MD,** Alfred Koenigsrainer, MD,†† Nikolas H. Stoecklein, MD,‡‡ Olaf Horstmann, MD,§§ Robert Grützmann, MD,‖‖ Andreas Imdahl, MD,¶¶ Daniel Svoboda, MD,## Christian Wittekind, MD,¶¶¶ Wolfgang Schneider, MD,### Klaus-Dieter Wernecke, PhD,*** and Peter M. Schlag, MD, PhD**

Introduction: The clinical impact of sentinel lymph node biopsy (SLNB) in colon cancer is still controversial. The purpose of this prospective multicenter trial was to evaluate its clinical value to predict the nodal status and identify factors that influence these results.

Methods: Colon cancer patients without prior colorectal surgery or irradiation were eligible. The sentinel lymph node (SLN) was identified intraoperatively by subserosal blue dye injection around the tumor. The SLN underwent step sections and immunohistochemistry (IHC), if classified free of metastases after routine hematoxylin and eosin examination.

Results: At least one SLN (median, n = 2) was identified in 268 of 315 enrolled patients (detection rate, 85%). Center experience, lymphovascular invasion, body mass index (BMI), and learning curve were positively associated with the detection rate. The false-negative rate to identify pN+ patients by SLNB was 46% (38 of 82). BMI showed a significant association to the false-negative rate ($P < 0.0001$), the number of tumor-involved lymph nodes was inversely associated. If only slim patients (BMI ≤24) were investigated in experienced centers (>22 patients enrolled), the sensitivity increased to 88% (14 of 16). Moreover, 21% (30 of 141) of the patients, classified as pN0 by routine histopathology, revealed micrometastases or isolated tumor cells (MM/ITC) in the SLN.

Conclusions: The contribution of SLNB to conventional nodal staging of colon cancer patients is still unspecified. Technical problems have to be resolved before a definite conclusion can be drawn in this regard. However, SLNB identifies about one fourth of stage II patients to reveal MM/ITC in lymph nodes. Further studies must clarify the clinical impact of these findings in terms of prognosis and the indication of adjuvant therapy.

(*Ann Surg* 2007;245: 858–863)

Sentinel lymph node (SLN) biopsy (SLNB) has been found to be highly effective in correctly predicting the nodal status for melanoma and breast cancer patients.[1,2] In contrast, the current evidence for SLNB in colon cancer is conflictive. On the one hand, there are few study groups, who reported a high predictive value of SLNB for the nodal status,[3,4] hypothesized an improved staging by detection of small tumor deposits as well as an increased yield of harvested lymph nodes[5,6] and reported a significant percentage of aberrant drainage outside the planned resection margins.[7] On the other hand, several recent studies could not confirm these results.[8–11] Due to the fact that different methods were used and most of the studies revealed low patient numbers, we deemed it necessary to initiate a multicenter study, that assures an adequate number of patients, a consistent detection technique and a standardized histopathologic examination. The primary endpoint was the sensitivity to detect nodal positive patients. Secondary endpoints were detection rate, negative prediction value, accuracy, and the rate of upstaging. Moreover, the study aimed at the identification of specific factors that may influence these parameters.

PATIENTS AND METHODS

Study Organization

The study was initiated and organized in the framework of the oncologic working group of the German Society of Surgery (CAO-V), headed by the Department of Surgery and Surgical Oncology of the Robert-Rössle Cancer Center, Charité, University Medicine Berlin.

Reprints: Peter M. Schlag, MD, Department of Surgery and Surgical Oncology, Robert-Rössle-Klinik, Charité University Medicine Berlin, Campus Berlin-Buch, at HELIOS Klinikum Berlin, Lindenberger Weg 80, 13125 Berlin, Germany. E-mail: pmschlag@charite.de

ISSN: 0003-4932/07/24506-0858
DOI: 10.1097/01.sla.0000250428.46656.7e

Prior to the beginning of the study, an initial meeting was held for all interested cancer centers to introduce the method and discuss details of performance. Each center nominated a surgeon to be responsible for the conductance of the study. An instruction video was provided to any participating center and regular study meetings were held every 6 months during the study period (January 2003 to August 2005) for participating surgeons and pathologists.

Patients

Patients could be included in the study, if they were between 18 and 85 years of age and suffered from a histopathologically proven colon carcinoma (inclusion criteria). Written informed consent was mandatory for inclusion. Prior operation or irradiation of the colon/mesocolon, reduced physical condition (ASA IV), known allergy against dye and mental disorders rendered a study enrollment impossible (exclusion criteria). If the attempt was made to identify a SLN by blue dye injection during a colon resection with adequate lymphadenectomy, the patient was registered as "included" and a complete set of data recorded. In case no lymphadenectomy was performed, the patient was considered as "drop out" and no data were registered. The study was approved by the local Human Investigations Committee (Committee of Ethics) and covered by an appropriate insurance.

Study Design and Endpoints

The study was designed as a controlled prospective trial.

The primary endpoint was defined as the sensitivity to identify nodal positive patients by SLNB and the corresponding false-negative rate.

Secondary endpoints were detection rate, negative predictive value, accuracy, up-staging by focused analysis of the SLN, the rate of aberrant drainage, and the influence of various factors on sensitivity and detection rate (patient characteristics, features of the primary tumor, learning curve, and center experience).

Methods

After laparotomy (respectively placing of the trocars in laparoscopic technique) and mobilization of the tumor-bearing part of the colon, 2 to 4 mL Patent Blue dye were injected into the subserosal layer immediately adjacent to the tumor in 4 portions. The blue stained lymph node(s) that appeared within the first 10 minutes after injection was (were) tagged with a suture. The subsequent resection of the tumor was performed as a standardized radical hemicolectomy (left or right) or transverse colon resection.

After resection of the specimen, the tagged lymph node(s) was (were) excised and separately processed to further examination as sentinel lymph nodes (SLN). Thereafter, as many non-SLN lymph nodes as could be identified were dissected from the specimen (aiming at a minimum of 12 lymph nodes as recommended by the UICC/AJCC).

Lymph nodes up to 10 mm in diameter were bivalved. Larger lymph nodes were grossly sectioned in slices up to maximally 2 mm and processed to paraffin blocks for hematoxylin and eosin staining.

If none of the lymph nodes processed in this manner revealed metastases, the SLN underwent stepwise sections of 250 μm distance each until sampled completely. At each level, at least 2 serial sections were cut at 5 μm thickness and one of them was separated for immunohistochemical staining. In case no tumor cells were found by hematoxylin and eosin staining, at least 4 serial sections per lymph node were stained by immunohistochemistry (pan-cytokeratin antibody MNF116 visualized with streptavidin-AP, DAKO, Germany). Uncertain findings after hematoxylin and eosin staining were also clarified by immunohistochemistry. To exclude false-positive results by staining of perifollicular reticulum cells or plasma cells, CK-positive cells were only considered as tumor cells if they revealed unequivocal cytomorphological criteria of a tumor cell on double staining with hemalaun.

Based on the simplified model of spheric configurated metastases, this approach enables the pathologist to identify tumor deposits up to a diameter of 0.25 mm with a probability of 100%, tumor deposits up to a diameter of 0.1 mm with a probability of 50%.[12] Tumor cell deposits larger than $>$0.2 mm but smaller than 2 mm in diameter were classified as micrometastases (MM). Tumor cell clusters up to a diameter of 0.2 mm or single CK-positive cells were classified as "isolated tumor cells" (ITC) according to Hermanek et al[13] and the UICC/AJCC.[14]

Statistics

The following definitions were used for calculations:

Detection rate (%):

$$\frac{\text{Number of patients with successfully retrieved SLN} \times 100}{\text{Number of patients enrolled}}$$

Sensitivity (%):

$$\frac{\text{Number of patients with a tumor involved SLN} \times 100}{\text{Number of patients with macrometastases in any lymph node}}$$

False-negative rate (%):
100%-Sensitivity

Negative predictive value (%):

$$\frac{\text{Number of nodal negative patients} \times 100}{\text{Number of nodal negative patients} + \text{number of false-negative patients}}$$

Accuracy (%):

$$\frac{\text{Number of patients with correct prediction of the nodal status} \times 100}{\text{Number of patients enrolled}}$$

Based on the assumption of a (true) sensitivity SE = 95% and an intended exactness $\Delta = \pm 10\%$ of the corresponding estimation, the required number of patients was n = 232 to obtain a statistical power of 80%.

All (primary and secondary) endpoints were initially analyzed by exploratory statistical procedures. The association of tumor and patients' characteristics to detection rate and sensitivity was tested by univariate analysis using the exact χ^2 test for categorical variables and the exact linear-by-linear association test for ordered categories,[15] respectively. Thereby, the indicator variables "SLN found" versus "SLN not found" and "result (true) positive" versus "result

false negative" were representative for detection rate and sensitivity, respectively. For the association analyses, continuous variables were allocated into classes of equal size. Parameters that revealed a significant influence in univariate analysis were further analyzed by multivariate logistic regression to adjust the results for essential clinical parameters. P values <0.05 were considered significant.

If metrically scaled parameters like the body mass index (BMI) or center experience (number of enrolled patients) were found to be significantly associated with detection rate or sensitivity, a receiver operating characteristic (ROC) curve analysis with respect to the indicator variables was performed. The maximal sum of sensitivity + specificity provides that cutoff level of the examined parameter, which separates the collective of patients with the highest significance.

"Center experience" was defined as the number of patients enrolled by each center. For calculations concerning the "learning curve," patients of the different centers were assigned to groups according to the order of their enrollment by each center (eg, all patients that were first enrolled by each center were included in one group, all patients that were second, etc). Subsequently, the different groups were compared in terms of detection rate, sensitivity and upstaging.

After approval of the study by the local ethics committee, 19 German university centers enrolled 315 patients, the median number of patients provided per center was 10 (range, 1–85). A median number of 20 (range, 4–79) lymph nodes per patient was examined.

Data of the 315 enrolled patients, procedures, and tumor stages are summarized in Table 1.

RESULTS

Detection Rate

At least one SLN was found in 268 of 315 (85%) patients undergoing the SLNB procedure. No adverse reaction after blue dye injection was recorded.

TABLE 1. Characteristics of Patients and Interventions

General Parameter	Value (range)
Total no. (%) patients	315 (100)
Female [no. (%)]	129 (41)
Male [no. (%)]	186 (59)
Age (yr) [median (range)]	67 (21–89)
Open surgery [no. (%)]	293 (93)
Laparoscopic surgery	22 (7)
No. examined lymph nodes per patient [median (range)]	20 (7–79)
Length of stay in hospital (days) [median (range)]	14 (5–116)
Complication rate	
Minor* (%)	13
Major† (%)	10
Overall (%)	24
Hospital mortality (%)	6

*No surgical reintervention necessary.
†Surgical reintervention necessary.

A significant association to the detection rate was found for center experience ($P = 0.027$), learning curve ($P = 0.042$), BMI ($P = 0.037$), and lymphovascular invasion ($P = 0.021$). No significant association was encountered for tumor infiltration depth (pT) ($P = 0.090$), age, sex, vascular invasion, number of involved lymph nodes, total number of lymph nodes examined, and tumor localization.

To determine the cutoff level for "center experience," a ROC-curve analysis was performed. It revealed 22 enrolled patients as cutoff level, corresponding to a detection rate of 76.4% for centers with up to 22 patients enrolled versus a detection rate of 91.0% for centers with more than 22 patients enrolled ($P < 0.0001$).

The cutoff level calculation for the BMI revealed 25 kg/m^2 corresponding to a detection rate of 92,5% for patients with a BMI $\leq$25 kg/m^2 versus a detection rate of 80% for patients with a BMI $>$25 kg/m^2 ($P = 0.003$).

The multivariate logistic regression analysis yielded the most significant impact for center experience ($P < 0.0001$), followed by lymphovascular invasion ($P = 0.004$) and BMI (categorical: $\leq$25 vs. $>$25 kg/m^2; $P = 0.002$).

Sensitivity of SLNB to Predict Macrometastases

Overall, 82 of 268 patients (31%) were found to have macrometastases in the SLN or non-SLN and, thus, were considered as nodal positive (pN1 or pN2). In 44 of these patients, the SLN was infiltrated by tumor cells resulting in a sensitivity of 54% (44 of 82), whereas in the remaining 38 patients the SLN was free of tumor cells, but metastases were found in non-SLN, resulting in a false-negative rate of 46% (38 of 82). In all but 6 of these 38 patients, step sections and immunohistochemistry were used to for SLN examination to exclude the presence of even very small tumor deposits.

A significant, inverse association to sensitivity was found for the BMI ($P = 0.009$), whereas the number of involved lymph nodes revealed a positive association ($P = 0.033$).

No significant association was encountered for age, sex, lymphovascular/vascular invasion, grading, pT classification, total number of examined lymph nodes, center experience, learning curve, or location of the tumor.

To determine the cutoff level for the BMI, a ROC-curve analysis was performed. It revealed a BMI of 24 kg/m^2 as cutoff level, corresponding to a sensitivity of 80% for patients with a BMI up to 24 kg/m^2 versus a sensitivity of 42% for patients with a BMI $>$24 kg/m^2 ($P < 0.0001$).

The multivariate regression analysis revealed the BMI (categorical: $\leq$24 vs. $>$24 kg/m^2) to have the most significant impact on sensitivity ($P < 0.0001$), followed by the number of tumor-involved lymph nodes ($P = 0.024$).

Sensitivity After Optimized Conditions

If optimized conditions were provided by a selection of patients with a BMI $\leq$24, who underwent SLNB in an experienced center ($>$22 enrolled patients), the sensitivity to detect macrometastases would have been 88% (14 of 16).

Identification of Micrometastases and Isolated Tumor Cells by Step Sectioning and IHC of the SLN ("Upstaging")

To define the rate of "upstaging," the proportion of patients was calculated, that was found to reveal micrometastases or isolated tumor in the SLN by step sectioning and IHC, but has been classified as N0 by routine hematoxylin and eosin-staining. A total of 141 of 186 patients classified as nodal negative by routine hematoxylin and eosin staining underwent step sections and immunohistochemistry of the SLN. Thirty of these patients revealed micrometastases (n = 7) or isolated tumor cells (ITC, n = 23), resulting in an overall upstaging rate of 30 of 141 (21.3%). In the clinically important subgroup of stage II patients, the upstaging rate was 24.2% (21 of 91).

Accuracy

The accuracy to predict the nodal status by SLNB was 85.8% (230 of 268).

Negative Predictive Value

The negative predictive value for the prediction of the absence of macrometastases was 80.0% (148 of 186).

Aberrant Lymphatic Drainage

Aberrant drainage, defined as lymphatic drainage to a lymph node that is located outside the planned resection margins, was found only in 5 patients (1.6%) without proof of metastases in the aberrant nodes.

An overview of the results is depicted in Table 2.

DISCUSSION

On the one hand, the results of our study showed that SLNB using the blue dye method is still unable to predict the nodal status with a clinically acceptable accuracy, at least in a multicenter setting: Despite a high detection rate, the overall sensitivity to identify macrometastases was low (54%) and thus confirmed the results of several smaller trials in this regard.[11,16,17]

On the other hand, however, detection rate and sensitivity were significantly influenced by patient- and disease-specific factors, in particular BMI, center experience, and lymphovascular invasion. The important relevance of these factors indicated that technical problems like visibility of the lymph channels in obese patients (BMI >24 kg/m^2) and the problem of the learning curve need to be resolved before a definite conclusion in regard to the accuracy of the method can be drawn.

Further studies must clarify whether or not technical developments like alternative dyes with a better visibility in fatty tissue or better rheologic properties of the tracer will be able to reduce the false-negative rate. The finding that a selection of patients (BMI ≤ 24 kg/m^2, treatment in experienced centers) yielded a sensitivity of 88% (14 of 16), which is comparable to the findings in breast cancer, may underline the importance of the technical aspects.

No association was found between the total number of lymph nodes examined and the false-negative rate ($P = 0.136$). This state of affairs further underlines that there are other, additional factors to influence the probability to find metastases in non-SLN than the radicality of the lymph node resection and the quality of the histopathologic examination.

Although a rare event, lymph node recurrence is assumed to emerge from lymphatic drainage outside the planned resection margins.[18] Bilchik et al hypothesized that SLNB could detect this so-called "aberrant lymphatic drainage" in a significant percentage of patients and, thus, help to prevent locoregional tumor recurrence.[7] Unfortunately, in our study, aberrant drainage was found only in 5 patients (1.6%) without proof of metastases in the aberrant nodes. Thus, it is unlikely that SLNB using the blue dye method can alter the surgical management in colon cancer by the identification of unpreviewed lymphatic drainage in a clinically relevant percentage of patients.

However, the present study revealed micrometastases or isolated tumor cells in the SLN of more than 20% of patients classified initially as pN0 by routine histopathologic examination of all lymph nodes (SLN and non-SLN). Our results thereby confirmed the findings of other studies using a comparable examination technique.[19–22] However, these findings raise the question of whether the SLN exhibits a higher probability to contain small tumor deposits than non-SLN or whether a random lymph node selection would provide the same percentage of upstaging as obtained by SLNB. A large number of studies already addressed the identification of MM/ITC cells by focused examination of all lymph nodes without using SLNB,[23–26] but using special techniques like the "fat clearance technique" to increase the yield of lymph nodes.[27] In these studies, the percentage of patients with MM/ITC in their lymph nodes varied between 18% and 76%.[23–26,28–31] As the definition of a "positive finding" and the technique of histopathologic assessment varied between many studies, the results are difficult to compare. Recently, however, Turner et al[32] and our group[21] examined SLN and non-SLN in the same manner by step sections and immunohistochemistry. It was found that the SLN bore a significantly higher probability to contain MM/ITC than non-SLN and is highly predictive not only for the presence, but also for the absence of MM/ITC (sensitivity and negative predictive value $>90\%$). Because of the frequent coincidence of MM/ITC in SLN and non-SLN,[32,21] it is unlikely that these findings were the result of artificial epithelial displacement by technical manipulations as hypothesized

TABLE 2. Overview of Results From Sentinel Lymph Node Biopsy in 315 Colon Cancer Patients

Study Population (N)	Detection Rate	Sensitivity	Upstaging (overall)	Upstaging Stage II	Negative Predictive Value	Accuracy	Aberrant Drainage
315	85% (268/315)	54% (44/82)	21% (30/141)	24% (21/91)	80% (146/186)	86% (230/268)	1.6% (5/315)

by Diaz et al.[33] These 2 studies are in contrast to the previously published study of Redston et al,[34] which reported no difference between SLN and non-SLN in regard to the frequency of MM/ITC-involvement. Among other criteria, however, the results of this study seemed to be negatively influenced by a very small number of lymph nodes, that underwent intensified examination (n = 101) and the lack of morphologic criteria to identify tumor cells. Thus, the currently available data nevertheless suggest that SLNB is a relevant method to identify MM/ITC in lymph nodes of patients conventionally classified as pN0 by routine hematoxylin and eosin examination.

The biologic background and the prognostic significance of these findings are, however, still unclear.[23–26,28–31] Therefore, it is of major importance to clarify the prognostic role of these findings but also to compare its clinical significance with new methods like molecular profiling of the primary tumor.[35] SLNB, at least, could be a practicable and less time-consuming tool to address these questions in a sufficiently large study.

CONCLUSION

Our study showed that SLNB using the blue dye technique still fails to have a significant impact on surgery or routine nodal staging. However, as factors like BMI and center experience influenced the results significantly, technical problems have to be resolved before a definite conclusion can be drawn, whether or not SLNB is able to identify macrometastases with a clinically sufficient accuracy. On the other hand, SLNB enables a simple identification of a subpopulation of more than 20% of stage II patients, revealing MM/ITC in their lymph nodes. As the prognostic role of these finding still remains to be clarified, SLNB may serve as a diagnostic tool to clarify this question.

AFFILIATIONS

From the *Department of Surgery and Surgical Oncology at "Robert-Rössle-Klinik," Charité University Medicine Berlin, Campus Buch at Helios-Klinikum Berlin; †Department of Surgery, "Klinikum rechts der Isar," Technical University of Munich; ‡Department of Abdominal-, Thoracic-, and Vascular Surgery, University Clinic of Leipzig; §Department of Surgery, University Clinic of Erlangen; ‖Department of General, Vascular and Thoracic Surgery, Charité University Medicine Berlin, Campus Benjamin Franklin, Berlin; ¶Department of General Surgery, University Clinic of Münster; #Department of General and Thoracic Surgery, University Clinic of Schleswig-Holstein, Campus Kiel; **Department of Surgery at "Klinikum Groβhadern," Ludwig-Maximilians-University Munich; ††Department of General, Visceral and Transplantation Surgery, University Clinic of Tübingen; ‡‡Department of General and Visceral Surgery, University Clinic of Düsseldorf; §§Department of General Surgery, University Clinic of Göttingen; ‖‖Department of Visceral, Thoracic and Vascular Surgery, University Clinic of Dresden; ¶¶Department of General and Visceral Surgery, University Clinic of Freiburg; ##University Clinic of Marburg, Department of Visceral, Thoracic and Vascular Surgery, Marburg; ***Institute of Medical Biometry, Charité University Medicine Berlin; †††Institute of Pathology, "Klinikum rechts der Isar," Technical University of Munich; ‡‡‡University Clinic of Erlangen, Institute of Pathology, Erlangen; §§§Institute of Pathology, University Clinic of Münster; ‖‖‖Institute of Pathology, University Clinic of Schleswig-Holstein, Campus Kiel; ¶¶¶Institute of Pathology, University Clinic of Leipzig; and ###Institute of Pathology, Robert Rössle Klinik Charité at Helios-Klinikum Berlin; with collaboration of the Department of Visceral, Thoracic and Vascular Surgery, University Clinic of Marburg, Department of General, Visceral Thoracic and Vascular Surgery, University Clinic of Greifswald, Department of General, Visceral and Transplantation Surgery, Charité University Medicine Berlin, Campus Virchow-Klinikum, Department of General, Visceral and Transplantation Surgery, Medical Highschool Hannover. All institutions are located in Germany.

REFERENCES

1. Thompson JF. The Sydney Melanoma Unit experience of sentinel lymphadenectomy for melanoma. *Ann Surg Oncol*. 2001;8:44–47.
2. Veronesi U, Paganelli G, Viale G, et al. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. *N Engl J Med*. 2003;349:546–553.
3. Bilchik AJ, Giuliano AE, Essner R, et al. Universal application of intraoperative lymphatic mapping and sentinel lymphadenectomy in solid neoplasms. *Cancer J Sci Am*. 1998;4:351–358.
4. Saha S, Wiese D, Badin J, et al. Technical details of sentinel lymph node mapping in colorectal cancer and its impact on staging. *Ann Surg Oncol*. 2000;7:120–124.
5. Bilchik AJ, Nora DT, Sobin LH, et al. Effect of lymphatic mapping on the new tumor-node-metastasis classification for colorectal cancer. *J Clin Oncol*. 2003;21:668–672.
6. Saha S, Seghal R, Patel M, et al. A multicenter trial of sentinel lymph node mapping in colorectal cancer: prognostic implications for nodal staging and recurrence. *Am J Surg*. 2006;191:305–310.
7. Bilchik AJ, Saha S, Tsioulias GJ, et al. Aberrant drainage and missed micrometastases: the value of lymphatic mapping and focused analysis of sentinel lymph nodes in gastrointestinal neoplasms. *Ann Surg Oncol*. 2001;8:82–85.
8. Read T, Fleshman JW, Caushaj PF. Sentinel lymph node mapping for adenocarcinoma of the colon does not improve staging accuracy. *Dis Colon Rectum*. 2005;48:80–85.
9. Fleig BW, Curley S, Lucci A, et al. A caution regarding lymphatic mapping in patients with colon cancer. *Am J Surg*. 2001;182:707–712.
10. Merrie AEH, van Rij AM, Phillips LV, et al. Diagnostic use of the sentinel node in colon cancer. *Dis Colon Rectum*. 2001;44:410–417.
11. Bertagnolli M, Miedema B, Redston M, et al. Sentinel node staging of resectable colon cancer: results of a multicenter study. *Ann Surg*. 2004;240:624–630.
12. Meyer JS. Sentinel lymph node biopsy: strategies for pathologic examination of the specimen. *J Surg Oncol*. 1998;69:212–218.
13. Hermanek P, Hutter RV, Sobin LH, et al. International Union Against Cancer: classification of isolated tumor cells and micrometastasis. *Cancer*. 1999;86:2668–2673.
14. Sobin LH, Wittekind C. *TNM Classification of Malignant Tumors*. New York: Wiley-Liss, 2002.
15. Agresti A, ed. *Categorical Data Analysis*. New York: Wiley, 1990.
16. Broderick-Villa G, Amr D, Haigh PI, et al. Ex vivo lymphatic mapping: a technique to improve pathologic staging in colorectal cancer. *Am Surg*. 2004;70:937–941.
17. Bell SW, Mourra N, Flejou JF, et al. Ex vivo sentinel lymph node mapping in colorectal cancer. *Dis Colon Rectum*. 2005;48:74–79.
18. Read TE, Mutch MG, Chang BW, et al. Locoregional recurrence and survival after curative resection of adenocarcinoma of the colon. *J Am Coll Surg*. 2002;195:33–40.
19. Wong JH, Steineman S, Caldena C, et al. Ex vivo sentinel node mapping in carcinoma of the colon and rectum. *Ann Surg*. 2001;233:512–521.
20. Bembenek A, Schneider U, Gretschel S, et al. Optimization of staging in

colon cancer using sentinel lymph node biopsy. *Der Chirurg*. 2005;75: 761–766.
21. Bembenek A, Schneider U, Gretschel S, et al. Detection of lymph-node micrometastases and isolated tumor cells in sentinel and non-sentinel lymph nodes of colon-cancer patients. *World J Surg*. 2005;29:1172–1175.
22. Bilchik AJ, Nora D, Tollenaar AEM, et al. Ultrastaging of early colon cancer using lymphatic mapping and molecular analysis. *Eur J Cancer*. 2002;18:977–985.
23. Rosenberg R, Friederichs J, Gertler R, et al. Prognostic evaluation and review of immunohistochemically detected disseminated tumor cells in peritumoral lymph nodes of patients with pN0 colorectal cancer. *Int J Colorectal Dis*. 2004;19:430–437.
24. Fisher ER, Colangelo L, Wieand S, et al. Lack of influence of cytokeratine-positive mini micrometastases in 'negative node' patients with colorectal cancer: findings from the National Surgical Adjuvant Breast and Bowel Projects Protocols R-01 and C-01. *Dis Colon Rectum*. 2003;46:1021–1026.
25. Noura S, Yamamoto H, Miyake Y, et al. Immunohistochemical assessment of localization and frequency of micrometastases in lymph nodes of colorectal cancer. *Clin Cancer Res*. 2002;8:759–767.
26. Yasuda K, Adachi Y, Shiraishi N, et al. Pattern of lymph node micrometastasis and prognosis of patients with colorectal cancer. *Ann Surg Oncol*. 2001;8:303–304.
27. Haboubi NY, Abdalla SA, Amini S, et al. The novel combination of fat clearance and immunohistochemistry improves prediction of the outcome of patients with colorectal carcinomas: a preliminary study. *Int J Colorectal Dis*. 1998;13:99–102.
28. Isaka N, Nozue M, Doy M, et al. Prognostic significance of perirectal lymph node micrometastases in Dukes' B rectal carcinoma: an immunohistochemical study by CAM5. 2. *Clin Cancer Res*. 1999;5:2068.
29. Greenson JK, Craig EI, Rice R, et al. Identification of occult micrometastases in pericolic lymph nodes of Dukes' B colorectal cancer patients using monoclonal antibodies against cytokeratin and CC49. *Cancer*. 1994;73:563–569.
30. Tschmelitsch J, Klimstra DS, Cohen AM. Lymph node micrometastases do not predict relapse in stage 2 colon cancer. *Ann Surg Oncol*. 2000;7:601–608.
31. Broll R, Schauer V, Schimmelpennig H, et al. Prognostic relevance of occult tumor cells in lymph nodes of colorectal carcinomas. *Dis Colon Rectum*. 1997;40:1465–1471.
32. Turner RR, Nora DT, Trocha SD, et al. Colorectal carcinoma nodal staging: frequency and nature of cytokeratin-positive cells in sentinel and nonsentinel lymph nodes. *Arch Pathol Lab Med*. 2003;127:673–679.
33. Diaz NM, Cox CE, Ebert M, et al. Benign mechanical transport of breast epithelial cells to sentinel lymph nodes. *Am J Surg Pathol*. 2004;28: 1641–1645.
34. Redston M, Compton CC, Miedema BW, et al. Analysis of micrometastatic disease in sentinel lymph nodes from resectable colon cancer: results of cancer and Leukemia Group B Trial 80001. *J Clin Oncol*. 2006;24:878–883.
35. Berger AV, Davis DW, Tellez C, et al. Automated quantitative analysis of activator protein-2alpha subcellular expression in melanoma tissue microarrays correlates with survival prediction. *Cancer Res*. 2005;65: 11185–11192.

